Role of nitric oxide in airway remodelling

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ABSTRACT

Airway remodelling, which is manifested by thickening of bronchial wall, is an important causative factor of bronchial hyper-responsiveness in asthma. The pathophysiological mechanism of airway remodelling is not clear. In the present study we evaluated the relationship between nitric oxide (NO) generation and airway wall thickening in patients with chronic asthma. As a marker of NO production, the levels of nitrite/nitrate were measured in induced sputum, and bronchial wall thickening was measured by high-resolution computed tomography. Sputum concentrations of nitrite/nitrate were significantly increased in asthmatic patients compared with controls. The ratio of airway wall thickness to lumen diameter was significantly correlated with the sputum concentration of nitrite/nitrate. Although statistical correlation does not prove causation, this finding suggests that NO may play a key role in the pathogenesis of airway remodelling.

INTRODUCTION

Bronchial asthma is a chronic inflammatory disease characterized by intermittent airflow obstruction and airway hyper-responsiveness [1]. Although acute exacerbation of the disease responds to the use of β-adrenergic bronchodilators, in the chronic disease a non-reversible component of hyper-reactivity persists that is unresponsive to β-adrenergic agonist therapy. Recent studies have suggested that airway remodelling, leading to thickening of the bronchial wall, is responsible for this underlying irreversible airway hyper-reactivity [1].

The mechanism of this airway remodelling is poorly understood; cytokines and growth factors, such as tumour necrosis factor α, transforming growth factor and platelet-derived growth factor, have been proposed to play some role [1]. Nitric oxide (NO) has also been reported to induce cellular and molecular events relevant to tissue remodelling. NO can modulate airway smooth muscle tone, bronchial oedema and bronchial inflammation, and is known to be increased in the exhaled air of asthmatic patients [2,3]. Whether NO plays a role in airway remodelling has not been addressed previously. To clarify this point, in the present study we evaluated the relationship between NO production and bronchial thickening in chronic asthmatic patients.

METHODS

 Patients
The study population comprised eight stable patients (six males and two females; mean age 55.5 years) with chronic bronchial asthma, and five healthy volunteers. The

Key words: bronchial asthma, nitric oxide, remodelling.

Abbreviations: CT, computed tomography; FEV1, forced expiratory volume in 1 s; $T_{aw}/D_L$ ratio, ratio of airway wall thickness to bronchial lumen diameter; VC, vital capacity.

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A diagnosis of asthma was made according to the diagnostic criteria of the American Thoracic Society [4]. Pulmonary function tests were performed using a volume-type spirometer (Chestac-55V; Chest MI Co., Tokyo, Japan). The mean value of the predicted vital capacity (VC) was 82.5±2.0%, and the mean value of the forced expiratory volume in 1 s (FEV₁) to VC was 63.5±4.5% when considering all asthmatic patients. Healthy subjects had no history of asthma or respiratory symptoms, a VC of > 80% of predicted value and a FEV₁/VC ratio of > 80%. Informed consent was obtained from all subjects before beginning the study. The study was approved by the Ethics Committee of our University and was carried out following the principles of the Declaration of Helsinki.

**Sputum induction**

Sputum was induced in all subjects by inhalation of increasing concentrations of hypertonic saline (3, 4 and 5%, w/v) for 10 min each using an ultrasonic Omuron NE-U12 nebulizer (Omuron Co., Tokyo, Japan) as previously described [5]. At the end of the inhalation, the subjects rinsed their mouth and blew their nose to avoid contamination with saliva and postnasal drip. Then they coughed sputum into a sterile container, and the portions free of salivary contamination were placed in a 50 ml polystyrene tube. This selected portion of the sputum was then diluted 10-fold with PBS (0.5 M NaH₂PO₄, 0.15 M NaCl, pH 7.4) and centrifuged at 200 g for 10 min. The supernatants were divided into aliquots and stored at −80°C until use. A 1 ml portion of 0.2% (w/v) freshly prepared dithiothreitol (Wako Pure Chemical Industry, Osaka, Japan) was added to the cell pellet, followed by incubation at 37°C for 20 min to ensure complete dissolution of the disulphide bonds of the mucus. The suspension was filtered through a 48 μm-pore-size nylon gauze to remove cell debris and mucus, and then centrifuged at 200 g for 10 min. The pellet was then resuspended in PBS and the cell differentials (percentages of neutrophils, lymphocytes, monocytes etc.) were determined on May–Grunwall Giemsa-stained cytospin preparations.

**Biochemical analyses and high-resolution computed tomography (CT)**

As markers of airway inflammation, sputum concentrations of total protein and eosinophilic cationic protein were measured using a dye-binding assay (Bio-Rad Laboratories, Hercules, CA, U.S.A.) and a radioimmunoassay (Pharmacia-Upjohn, Tokyo, Japan) kit respectively, in accordance with the manufacturer’s instructions. As an index of local NO production, levels of nitrite/nitrate in sputum were measured using a commercial colorimetric assay kit (Cayman Chemical, Ann Arbor, MI, U.S.A.). The interassay and intra-assay coefficients of variation were less than 10%.

High-resolution CT was performed using a GE 9800 CT scanner (General Electric Medical Systems, Milwaukee, WI, U.S.A.). All images were photographed and viewed at a window width of 2000 Housefield units and a window level of −700 Housefield units. The final interpretation of the high-resolution CT findings was obtained by consensus of two radiologists who were unaware of the patients’ backgrounds. In all patients, 1.5–2 mm-thick axial high-resolution CT was obtained at 0.5–1 cm intervals through the main, segmental and subsegmental bronchi of the upper lobes. The ratio of airway wall thickness to bronchial lumen diameter (\(T_{sw}/D_{b}\) ratio) was measured in all bronchial segments, and its average value was used as an index of airway remodelling.

**Statistical analysis**

All data are expressed as the mean ± S.E.M. The difference between the means of two variables was calculated using the Mann–Whitney U-test. The strength of the correlation between variables was analysed by the Pearson product moment. A \(P\) value of < 0.05 was considered statistically significant. Statistical analyses were carried out using the StatView 4.1 package software for the Macintosh (Abacus Concepts, Berkeley, CA, U.S.A.).

**RESULTS**

The percentage of eosinophils (59.6±12.6% compared with 0.3±0.2%; \(P < 0.01\)) and the concentrations of total protein (277.5±39.3 compared with 127.0±30.6 μg/ml; \(P < 0.02\)) and eosinophilic cationic protein (426.3±285.5 compared with 4.83±1.1 μg/l; \(P < 0.01\)) in induced sputum were significantly increased in asthmatic patients as compared with controls, suggesting that a marked inflammatory reaction had occurred in the airways of our patients. The percentage of macrophages and neutrophils was not significantly different between the asthmatic and control groups. The sputum concentration of nitrite/nitrate (11.3±1.3 compared with 6.1±1.6 μmol/l; \(P < 0.05\)) in patients with bronchial asthma than in healthy controls (Figure 1). The \(T_{sw}/D_{b}\) ratio was greater in asthmatic patients than in controls (0.32±0.06 compared with 0.12±0.01). The sputum concentration of nitrite/nitrate was positively and significantly (\(r = 0.9; P = 0.03\)) correlated with the \(T_{sw}/D_{b}\) ratio (Figure 2). Neither the sputum concentration of nitrites nor the \(T_{sw}/D_{b}\) ratio was correlated with the age of the patients. The \(T_{sw}/D_{b}\) ratio was not significantly correlated with the values of VC or the FEV₁/VC ratio.
The sputum concentration of nitrite/nitrate was significantly increased in patients with asthma as compared with healthy controls. This finding is in agreement with previous studies showing increased exhaled NO in patients with asthma [3]. The source of NO in the airways appears to be the bronchial epithelial cells and macrophages [6,7].

The significance of NO in asthma is unclear. Recent investigations have implicated NO in the mechanism of airway hyper-responsiveness [8]. It is well known that bronchial wall thickening, which results from airway remodelling, is an important causative factor of bronchial hyper-reactivity [9]. On this basis, we evaluated whether NO generation, measured as the sputum levels of nitrite/nitrate, is also associated with the thickness of airway walls. This latter parameter was measured by high-resolution CT, which has recently gained wide acceptance for the evaluation of hyper-responsiveness in asthmatic patients [9]. Our results showed that the sputum levels of nitrite/nitrate were significantly correlated with the $T_{aw}/D_{L}$ ratio, suggesting that NO may play a key role in the pathogenesis of airway remodelling; the results of previous studies support this assumption.

It has been shown previously that the inflammatory response in the airways of asthmatic patients is characterized by an abundant infiltration of mononuclear cells and eosinophils, mucus hypersecretion, and airway remodelling with smooth muscle hyperplasia and sub-epithelial fibrosis [1]. Secretion of NO from inflammatory cells and activated bronchial epithelial cells may be the cause of the increased concentration of NO in the exhaled air of asthmatic patients [6,7]. Sustained high levels of NO in the airway may induce increases in pulmonary blood flow and vascular permeability, and thus in plasma exudation and oedema [10,11]. Abnormally high levels of NO could also result in the accumulation of eosinophils and in exacerbated IgE-mediated responses in the airways by inducing overactivation of T helper type 2 lymphocytes [12]. Based on these findings, it is conceivable that, in asthmatic patients with a chronic clinical course, persistent pro-inflammatory activity caused by the increased levels of NO may culminate in the development of airway remodelling. At present there is no therapy available for airway remodelling. Further elucidation of the potential role of NO may offer a therapeutic target for this complication of bronchial asthma.

**REFERENCES**


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